REMARKS

Status of the Claims

After entry of the instant Amendment, claims 1-3 and 5-12 are pending in the present Application. Claims 1 and 2 are independent.

Claims 1-3 and 6-12 have been amended and claim 4 has been cancelled without prejudice or disclaimer of the subject matter contained therein. Support for amendments to the claims can be found throughout the Specification as filed. Claim 2 has been amended to recite limitations from claim 4. Reconsideration of this application, as amended, is respectfully requested.

Request for Entry of Response After Final Rejection

This response should be entered after final rejection because the claims should now be in condition for allowance.

In the event that this response does not place this application into condition for allowance, the Examiner is requested to enter this response because it places the application into better condition for appeal.

Priority under 35 U.S.C. § 119

Applicants thank the Examiner for acknowledging Applicants' claim for foreign priority under 35 U.S.C. § 119, and receipt of an English translation of the foreign priority document JP 2003-283703. However, due to a typographical error, the Office Action incorrectly indicates that JP 2003-283703 was filed in Japan on January 28, 1005. The actual filing date was July 31, 2003.

Information Disclosure Citations

In the Office Action it is stated that the Examiner was unable to determine whether the disclosure of WO 01/481148 is identical to the disclosure of JP 2003-518379. Applicants are herewith submitting a partial English translation of JP 2003-518379 including the Abstract and the claims for the Examiner's inspection.

Rejection under 35 U.S.C. § 102

Claims 1 and 4-6 remain rejected under 35 U.S.C. § 102(b) as being anticipated by Britt et al., "Autogenous tissue-engineered cartilage: evaluation as an implant material," Arch. Otolaryngol. Head Neck Surg., 1998, 124: 671-677 (hereinafter "Britt").

Claims 2-6 remain rejected under 35 U.S.C. § 102(b) as being anticipated by van Susante et al., "Linkage of chondroitin-sulfate to type I collagen scaffolds stimulates the bioactivity of seeded chondrocytes *in vitro*," Biomaterials, 2001, 22: 2359-2369 (hereinafter "van Susante").

As claim 4 has been cancelled its rejection is now moot. Applicants respectfully traverse the rejection of claims 1, 5 and 6 as being anticipated by Britt. The rejection of claims 2, 3, 5 and 6 as being anticipated by van Susante is also respectfully traversed.

At page 5 of the Office Action it is stated that "Applicant argues that both Britt and van Susante teach seeding cell suspension and not cell masses as required by the claims. This is not found persuasive because the claims do not require seeding cell masses." Applicants have amended the claims to clarify that claimed methods involve contacting at least one surface of a form with a plurality of spheroids to permit the spheroids to adhere to the surface, wherein has micropores smaller than the spheroids; and culturing the spheroids adhered to the surface under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface.

Britt does not disclose methods involving (a) culturing cells to form spheroids, (b) contacting at least one surface of a shaped carrier with a plurality of the spheroids to permit them to adhere to the surface, wherein the carrier has micropores smaller than the spheroids, and (c) culturing the adhered spheroids under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface of the shaped form, as in the claimed invention.

At pages 672 and 673, Britt teaches isolating chondrocytes from harvested cartilage, pelleting the chondrocytes, and suspending the chondrocytes in a small amount of Eagle minimum essential medium with 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid solution and penicillin-streptomycin. The chondrocyte suspension is then seeded onto polyglycolic acid-poly-L-lactic acid copolymer (PGA/PLA) mesh without first culturing the chondrocytes to form spheroids. Prior to seeding, the PGA/PLA mesh is wetted with ethanol to "allow better penetration of cell culture media and more uniform cell distribution." Britt teaches, at page 673, applying a sufficient volume of the chondrocyte suspension to the wetted PGA/PLA mesh to "achieve a cell concentration of 1 to

2 x $10^7/cm^3$ of template material [PGA/PLA mesh]." Further, at page 674, Britt states that "[u]niform chondrocyte seeding of the entire polymer mesh is a prerequisite for optimal graft development" [emphasis added].

As pointed out above, the claimed invention requires producing spheroids. At page 6 of the present Specification, it is disclosed that the micropores in the carrier are large enough to permit culture broth to pass through them, but too small to permit spheroids to pass through them.

The carrier of the claimed invention is not embedded within a layer of newly formed cartilage as with the PGA/PLA mesh within the cartilage/bone formed in methods disclosed by Britt. At page 674, Britt states that "flaws in graft morphological features [after *in vivo* culturing] are thought to result from the newly synthesized cartilage incompletely replacing the original polymer template. This can result from inadequacies in seeding the polymer template or from infiltration [*e.g.*, angiogenesis] of noncartilaginous tissues."

Thus, Britt cannot anticipate the claimed invention, because Britt does not teach first culturing cells to form spheroids and then contacting a surface of a shaped carrier with a plurality of the spheroids to permit them to adhere to the surface, where the carrier has micropores smaller than the spheroids, as in the claimed invention.

In view of the discussion above, Applicants respectfully request that the rejection of claims 1, 5 and 6 as being anticipated by Britt be withdrawn.

As with Britt, van Susante does not disclose methods involving (a) culturing cells to form spheroids, (b) contacting at least one surface of a shaped carrier with a plurality of the spheroids to permit them to adhere to the surface, wherein the carrier has micropores smaller than the spheroids, and (c) culturing the adhered spheroids under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface of the shaped form, as in the claimed invention.

At page 2361, van Susante teaches isolating chondrocytes and suspending them in Ham's F12/DMEM medium supplemented with 10% fetal calf serum and 0.1% penicillin/streptomycin. Insoluble type I collagen is used to prepare collagen matrices with and without chondroitin sulfate (CS) and the matrices are lyophilized. At page 2361, van Susante states that "[b]iopsy punches (6 mm) from the collagen matrices (with and without CS) were placed in a 96-well plate with the porous upper surface (air-side) facing up to facilitate penetration of the seeded cells into the pores." After addition of the suspension to the collagen sponges, they are gently centrifuged to further

permit the individual cells to penetrate into the pores.

Thus, van Susante cannot anticipate the claimed invention, because van Susante does not teach (as recited in the claims) first culturing chondrocytes to form spheroids and then contacting a surface of a shaped carrier with a plurality of the spheroids to permit them to adhere to the surface, where the carrier has micropores smaller than the spheroids.

In view of the discussion above, Applicants respectfully request that the rejection of claims 2, 3, 5 and 6 as being anticipated by van Susante be withdrawn.

Rejections under 35 U.S.C. § 103(a)

Claims 1-12 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Anderer et al., "*In vitro* engineering of human autogenous cartilage," Journal of Bone and Mineral Research, 2002, 17: 1420-1429 (hereinafter "Anderer"), in view of each of Tsutsumi et al., "Transforming growth factor-beta1 is responsible for maturation-dependent spontaneous apoptosis of cultured gastric pit cells," Exp. Biol. Med., 2002, 227: 402-411, Abstract, (hereinafter "Tsutsumi"), Long et al., WO 01/48148 (hereinafter "Long"), Hoffmann et al., "The T-box transcription factor Brachyury mediates cartilage development in mesenchymal stem cell line C3H10T1/2," J. Cell Sci., 2001, 115: 769-781 (hereinafter "Hoffman") and van Susante.

Claim 4 has been cancelled and its rejection is, therefore, moot. Applicants respectfully traverse the rejections of claims 1-3 and 5-12 as being unpatentable over Anderer, Tsutsumi, Long, Hoffmann, and van Susante.

As amended, claims 1 and 2 recite the following:

> culturing the spheroids adhered to the surface [of the carrier] under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface.

That is, in the claimed methods the spheroids fuse to each other on the surface of the carrier to produce new cartilage tissue on the surface. Anderer neither discloses nor suggests culturing the spheroids adhered to the surface of a carrier, but rather discloses a three-dimensional cell culture system without scaffolds by which human chondrocytes are cultured to become spheroids. (See Abstract, lines 4-5 of Anderer.) Anderer discloses spheroids cultured without scaffolds as producing "cartilage-like tissue." (See Abstract, lines 5-8 of Anderer.)

In order to increase the size of the spheroids, Anderer relies on the ability of aggregates to coalesce, but the fusion is carried out without a scaffold, resulting in the size of the cultured-spheroids being limited to less than 800-1000 μm in diameter. (See page 1426, right column, lines 1-8 of Anderer.) The cultured spheroids can be applied, for example, to osteoarthritic fissures to improve recovery. (See page 1427, right column, lines 8-11 of Anderer.)

In order to show that the cultured spheroids have the capacity to repair cartilage defects, Anderer's cartilage-explant spheroid co-culture system was used to place single spheroids on cartilage tissue of isolated human femoral condyles. (See page 1421, left column, line 56 to right column, line 3 of Anderer.) However, in Anderer's co-culture system, the fusion of the spheroids is not carried out after the spheroids are applied to the cartilage tissue. (See page 1424, left column, line 1 to right column, line 15 of Anderer.) Thus, Anderer neither discloses nor suggests culturing the spheroids adhered to the surface of a carrier under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface, as in the claimed invention.

Further, Tsutsumi, Long, Hoffmann and van Susante taken alone or together also do not disclose or suggest culturing the spheroids adhered to the surface of a carrier under conditions which induce the spheroids to fuse to each other and to produce new cartilage tissue on the surface, as recited in the claims.

Thus, the teachings of Anderer, Tsutsumi, Long, Hoffmann and van Susante taken alone or together do not teach each and every element of the claimed invention.

Further, the claimed invention has the advantage that the spheroids can be formed into a structure having a desired size and cell structure of interest. (See page 6, lines 22-24 of the present Specification.) That is, adhesion of the spheroids to the surface of the carrier promotes differentiation of the cells into cartilage, improvement of cell viability, and forming of a large cartilage structure, as shown in Examples 1 and 2. In contrast, the size of the "cartilage-like tissue" (*i.e.*, spheroids) taught by Anderer is smaller than 800-1000 μm in diameter, as discussed above. The thickness of the cartilage-like tissue formed by Anderer's methods would not be greater than the 800-1000 μm diameter of the spheroids.

Still further, Anderer teaches away from seeding chondrocyte suspensions on carriers/scaffolds (see Introduction), away from the use of antibiotics in culturing chondrocytes (see Abstract), away from culture medium without human serum (see Abstract), and away from

the use of growth factors in culturing chondrocytes (see Abstract). According to Anderer, at page 1426, "transferring a small amount of freshly isolated chondrocytes directly into a three-dimensional system leads to a proliferation stop (see Results), resulting in an insufficient cell number and density for tissue regeneration processes." Thus, Anderer teaches away from the methods of Tsutsumi (teaching use of TGF-β1), Long (teaching preparation of bone and culturing of spheroids without serum), Hoffmann (teaching use of BMP2 and culturing with antibiotics), and van Susante (teaching use of antibiotics and seeding suspension on scaffolds).

Describing her own work at page 1427, Anderer states that "[t]his aggregate culture system is a very effective method to generate in vitro cartilage-like tissue without using any scaffold, growth factor, or further additives. Using the aggregate culture technique supplemented only with autologous serum, chondrocytes formed a hyaline-like three-dimensional cell-matrix arrangement." Anderer does not teach carriers, as recited in the present claims.

At pages 9-10, Long states "[a] critical and distinguishing feature of the present invention are defined tissue culture conditions and factors resulting in the formation of bone cell spheroids. Sources of osteogenic or bone precursor cells and methods of isolating these precursor cells are also described." Long teaches away from culturing the bone cell spheroids in serum (pages 3-6; claims 1, 30, 31, 33-35, 37 and 38), while Anderer teaches the need for human serum for culturing cartilage cell spheroids (Abstract).

Hoffman teaches the need for BMP2 (bone morphogenetic protein 2, a growth factor) for inducing differentiation of murine mesenchymal stem cells to initiate chondrogenesis (Abstract). Anderer teaches away from the use of growth factors like BMP2. Hoffman also teaches the use of penicillin and streptomycin in cell culture (page 770), which Anderer also teaches away from.

Applicants respectfully point out that one of ordinary skill in the art would not be motivated to combine the teachings of the cited art, as proposed in the Office Action to arrive at the claimed invention, as the cited references teach away from each other.

Taken alone or together, Anderer, Tsutsumi, Long, Hoffmann, and van Susante do not teach methods of preparing cartilage comprising: (a) culturing stem cells to form spheroids; (b) shaping a carrier into a desired form; (c) contacting at least one surface of the form with a plurality of the spheroids to permit the spheroids to adhere to the surface, wherein the carrier has micropores smaller than the spheroids; and (d) culturing the spheroids adhered to the surface under conditions which induce the spheroids to fuse to each other and to produce new cartilage

tissue on the surface, as in the claimed invention.

In view of the discussion above, Applicants respectfully request that the rejections of claims 1-3 and 5-12 as being unpatentable over the teachings of Anderer, Tsutsumi, Long, Hoffmann, and van Susante be withdrawn.

CONCLUSION

All of the stated grounds of rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding rejections and that they be withdrawn. It is believed that a full and complete response has been made to the outstanding Office Action, and as such, the present application is in condition for allowance.

Should there by an outstanding matters that need to be resolved in the present application, the Examiner is respectfully requested to contact Stephanie A. Wardwell, Ph.D., Registration No. 48,025 at the telephone number of the undersigned below to conduct an interview in an effort to expedite prosecution in connection with the present application.

If necessary, the Director is hereby authorized in this, concurrent, and future replies to charge any fees required during the pendency of the above-identified application or credit any overpayment to Deposit Account No. 02-2448.

Dated: JUN 22 2010

Respectfully submitted,

By _____

Gerald M. Murphy, Jr.
Registration No.: 28977
BIRCH, STEWART, KOLASCH & BIRCH, LLP
8110 Gatehouse Road, Suite 100 East
P.O. Box 747
Falls Church, VA 22040-0747
703-205-8000

Attachment: Partial Translation of JP2003-518379